LITOMOSOIDES YUTAJENSIS N. SP., FIRST RECORD OF THIS FILARIAL GENUS IN A MORMOOPID BAT

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Summary:

Twenty-five bats were trapped in Yutaje (Amazonas, Venezuela) and examined for *Litomosoides* (Filarioidea: Onchocercidae). Of the nine recovered bat species, only *Pteronotus parnelli* was infected; it is a cave-dwelling species belonging to a family, Mormoopidae, which has not previously been included in the host range of the genus. The new species, *L. yutajensis* n. sp., has two median cephalic bosses covered with rugosities and differs from the 15 recognized species and subspecies from bats in several characters. Alike *L. molossi* Esslinger, 1973, *L. chandleri* Esslinger, 1973 and *L. chitwoodi* Bain, Guerrero, Rodriguez 2003, the new species has cuticular lateral bosses on the body. Eight of 10 *P. parnelli* were microfilaraemic, but only three had adult worms, showing that microfilariae survive longer than adults, which could lengthen the period of transmission. No infective larvae were detected in the following macronyssid mites: 58 *Ornithonyssus bacoti*, Ornithonyssinae, experimentally fed on microfilaraemic bats and dissected 15 days later, and a few *Radfordiella* sp., Macronyssinae, recovered from *P. parnelli*.

KEY WORDS : filariae, Onchocercidae, *Litomosoides*, new species, Chiroptera, biology, mites, Macronyssidae.

Résumé : *LITOMOSOIDES YUTAJENSIS* N. SP., PREMIÈRE FILAIRE DU GENRE RAPPORTÉE CHEZ UNE CHAUVE-SOURIS MORMOOPIDAE

Vingt-cinq chauves-souris ont été capturées à Yutaje (Amazonas, Venezuela) pour rechercher des Litomosoides *(Filarioidea : Onchocercidae). Sur les neuf espèces de chiroptères récoltées, seul* Pteronotus parnelli *était parasité ; c'est une espèce cavernicole de la famille des Mormoopidae, jusqu'à présent non répertoriée dans le spectre d'hôtes de* Litomosoides. L. yutajensis *n. sp. a deux mammelons céphaliques médians couverts d'aspérités et diffère des 15 espèces et sous-espèces connues chez les chauves-souris par plusieurs autres caractères. Comme* L. molossi *Esslinger, 1973,* L. chandleri *Esslinger, 1973, et* L. chitwoodi *Bain, Guerrero, Rodriguez 2003, la nouvelle espèce a des perles cuticulaires latérales sur le corps. Sept des 10* P. parnelli *capturés avaient des microfilaires sanguines et, parmi ceux-ci, trois n'avaient plus de filaires adultes montrant que les microfilaires pourraient ainsi allonger la période de transmission. Aucune larve infectante n'a été récoltée chez les Macronyssidae disséqués: 58* Bdellonyssus bacoti, *Ornithonyssinae, gorgés expérimentalement sur chauves-souris microfilariennes, et analysés 15 jours plus tard, et quelques* Radfordiella *sp., Macronyssinae, récoltés sur ces chauves-souris.*

MOTS CLÉS : *filaires, Onchocercidae, Litomosoides, nouvelle espèce, Chiroptera, biologie, acariens, Macronyssidae.*

INTRODUCTION

The filarial genus *Litomosoides* has several interesting features. It has been presented as an example of evolution by capture between bats, rodents and marsupials (Bain *et al.*, 1980; Brant & Gardner, 2000; Guerrero *et al.*, 2002). One of its species, *L. sigmodontis* Chandler, 1931, has become a much-used murine model for immunological analyses of filariases (Bain & Philipp, 1991; Martin *et al.*, 2001; Bain, 2002). Moreover, like several filariae of humans (Bandi *et al.*, 1999), this species harbours the endosymbiotic bacterium *Wolbachia*, which might revolutionize the concept of filarial pathologies and their therapy (Gencchi *et al.*, 1998; Hoerauf & Brattig, 2002; Saint Andre *et al.*, 2002). The phylogeny of *Wolbachia* contributes to the understanding of the evolution of onchocercid filariae (Bandi *et al.*, 2001; Casiraghi *et al.*, 2001).

Here we describe a new species of *Litomosoides* parasitic in Mormoopidae, a family of bats which has not previously been included in the host range of the genus. Some aspects of its biology are reported, and its relationships with other members of *Litomosoides* are considered.

MATERIALS AND METHODS

The study area was Yutaje (5° 36' 30" N, 66° 06' 51" W), Amazonas State, Venezuela. It is undisturbed tropical rain forest.

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Two mist nets were placed at ground level and opened in the evening just before dusk, for three hours. Trapped bats were placed individually in cotton bags. A blood sample of about 2 µl was taken by clipping a toenail; each blood sample was immediately examined microscopically for living microfilariae. When microfilariae were present, specimens of the macronyssid mite *Ornithonyssus bacoti* (Hirst 1913) were fed on the infected bat. Mites from a laboratory colony were kept in small tubes, 6 cm/1.5 cm, each containing 25 mites (Diagne *et al.*, 1990). For feeding, the mites were released on to a bat which had been gently immobilized in soft wire mesh and placed overnight above a container filled with water. In the morning the fed mites were recovered from the water and replaced in the tubes, as described by Bain *et al.* (2002). No incubator was available and the tubes were kept in a plastic box with moist cotton wool, at a temperature varying from 23 to 28° C; the box was opened daily for a few minutes to allow air to enter.

All bats were identified following the classification of Simons (1998). Any natural ectoparasites were recovered and a few mites were kept alive in tubes (as described for *O. bacoti*) for subsequent examination for filarial larvae.

When the bats were killed, thick blood films were prepared, and any adult filariae as well as nematodes from the digestive tract were recovered and fixed in hot 70 % alcohol. The morphological characters of the filariae were studied as described by Guerrero *et al.* (2002) and illustrations were prepared using a drawing tube. To stain the nuclei of microfilariae extracted from the uteri of fixed females, the vital stain Meldolan Blue was added to the aqueous medium; staining lasted for a few hours; the microfilarial sheath was also visualized with this technique.

RESULTS

MORPHOLOGICAL STUDY OF THE FILARIAE FROM THE MORMOOPID *PTERONOTUS PARNELLI* (GRAY 1843)

Filarial worms were located in the peritoneal cavity of *P. parnelli*, close to the dorsal wall, often under the liver. The studied material comprises the four males and five females recovered from the type host, specimen 102 CV, and a male worm, a posterior male extremity and a female obtained from bat 105 CV, and one of three males found in bat 117 CV, the other two having been fixed to examine for *Wolbachia*. Measurements are presented in Tables I and II and the morphology is illustrated in figures 1 and 2.

Head attenuated with two median bosses, both irregularly covered with tiny rugosities; in addition, two median cuticular groves often present. Four small externo-labial papillae arranged in a rectangle stretched dorso-ventrally, and two larger ventral cephalic papillae posterior to amphids. Buccal capsule of similar size in both sexes, posteriorly embedded in the oesophagus, slightly longer than broad (height/width ratio 1 to 1.6), with a thickened ring at its mid-length, more pronounced in females, giving the capsule a bell-like shape; frequent irregularities of the internal structure of the posterior wall (Fig. 2F); buccal cavity irregular in diameter, with one or two transverse grooves and often wider in the posterior third. Oesophagus with no distinct glandular part.

Male with three-four pairs of caudal papillae, the third pair being almost aligned and the last pair often reduced to one papilla. Spicules delicate; left spicule with handle, long proximal part of lamina devoid of longitudinally folded membranous alae, and thread-like distal part which, after dissection, appeared to be

Sample	**102 CV**	**102 CV**	**102 CV**	**102 CV**	**105 CV**
Measurements	Holotype	Paratype	Paratype	Paratype	
Length (mm)	17.00	17.75	15.50	17.15	16.95
Width max.	123	120	108	102	83
at nerve ring	50	50	52	50	52
at oe-in. junction	70	78	70	65	65
Buccal capsule H × W	13.2 × 11.4	16 × 10	11.5 × 11.5	14 × 12	16 × 10
Oesophagus L × W	619 × 22	637 × 25	569 × 19	581 × 21	546 × 23
Nerve ring	266	245	213	220	289
Tail L × W	150 × 40	160 × 39	145 × 33	154 × 34	126 × 37
Left spicule	257	279	303	no	288
handle	180	182	206	no	195
Right spicule	58	> 53	68	no	64
Area rugosa from cloaca	158	218	187	122	195
length	1,483	1,885	1,520	1,870	1,680
ridge height	1.0 – 1.5		1.0 – 2.1	1.0 – 1.6	0.7 – 1.5
ridges distance	4-7		7-8	6-7	6-9

Table I. – Measurements of males of *Litomosoides yutajensis* n. sp.

Sample	102 CV	102 CV	102 CV	102 CV	102 CV	105 CV
Measurements	Allotype	Paratype	Paratype	Paratype	Paratype	
Length (mm)	45.80	43.30	44.75	36.45	?	37.50
Width max.	195	202	195	202	202	205
at nerve ring	72	69	68	65	69	72
at oe-int. junction	120	110	118	126	160	150
Buccal capsule H × W	15 × 13	14.4 × 14.3	15.8 × 11.5	15.5 × 13	16 × 13	15.8 × 11.5
Oesophagus L × W	800 × 22	672 × 25	712 × 29	757 × 23	1,090 × 29	950 × 22
Nerve ring	257	260	246	242	307	296
Tail L × W	290 × 58	314 × 65	336 × 63	285 × 67	219 × 54	275 × 61
Vulva	500	513	480	535	450	565
Vagina L × W	90 × 75	76 × 56	94 × 72	84 × 76	116 × 72	98 × 67

Table II. – Measurements of females of *Litomosoides yutajensis* n. sp.

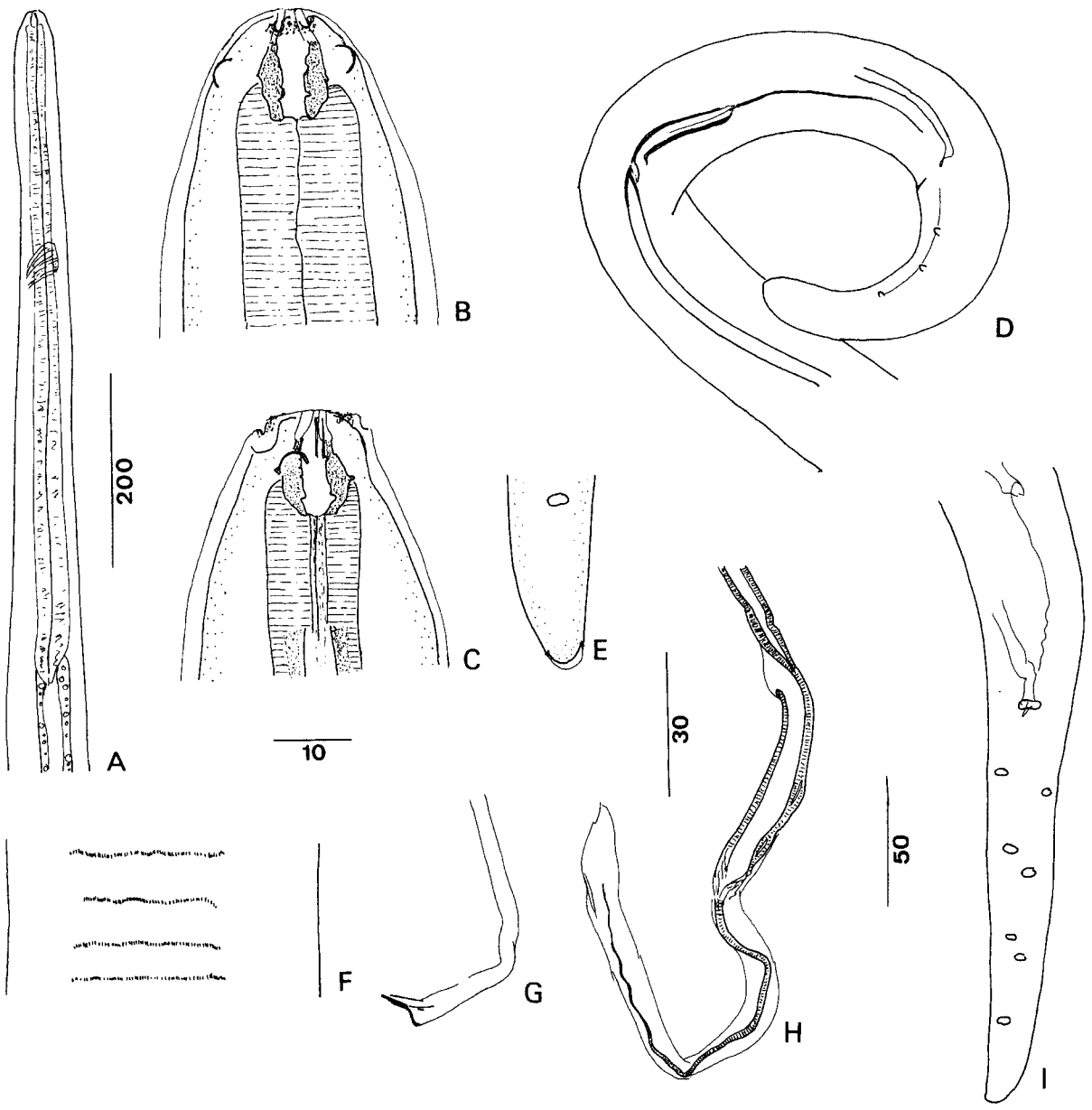

Fig. 1. – *Litomosoides yutajensis* n. sp. Male. A. Anterior region, left lateral view (holotype). B, C. Head, ventral and left lateral views, respectively (holotype). D. Posterior region, left lateral view. E. Tail extremity, ventral view. F. Area rugosa at mid-length, ventral view. G. Right spicule, lateral view, right lateral view. H. Lamina of left spicule (dissected out from spicular sheath). I. Tail, ventral view. Scales in µm: A, 200; B, C, 10; D, I, 50; E, F, G, H, 30.

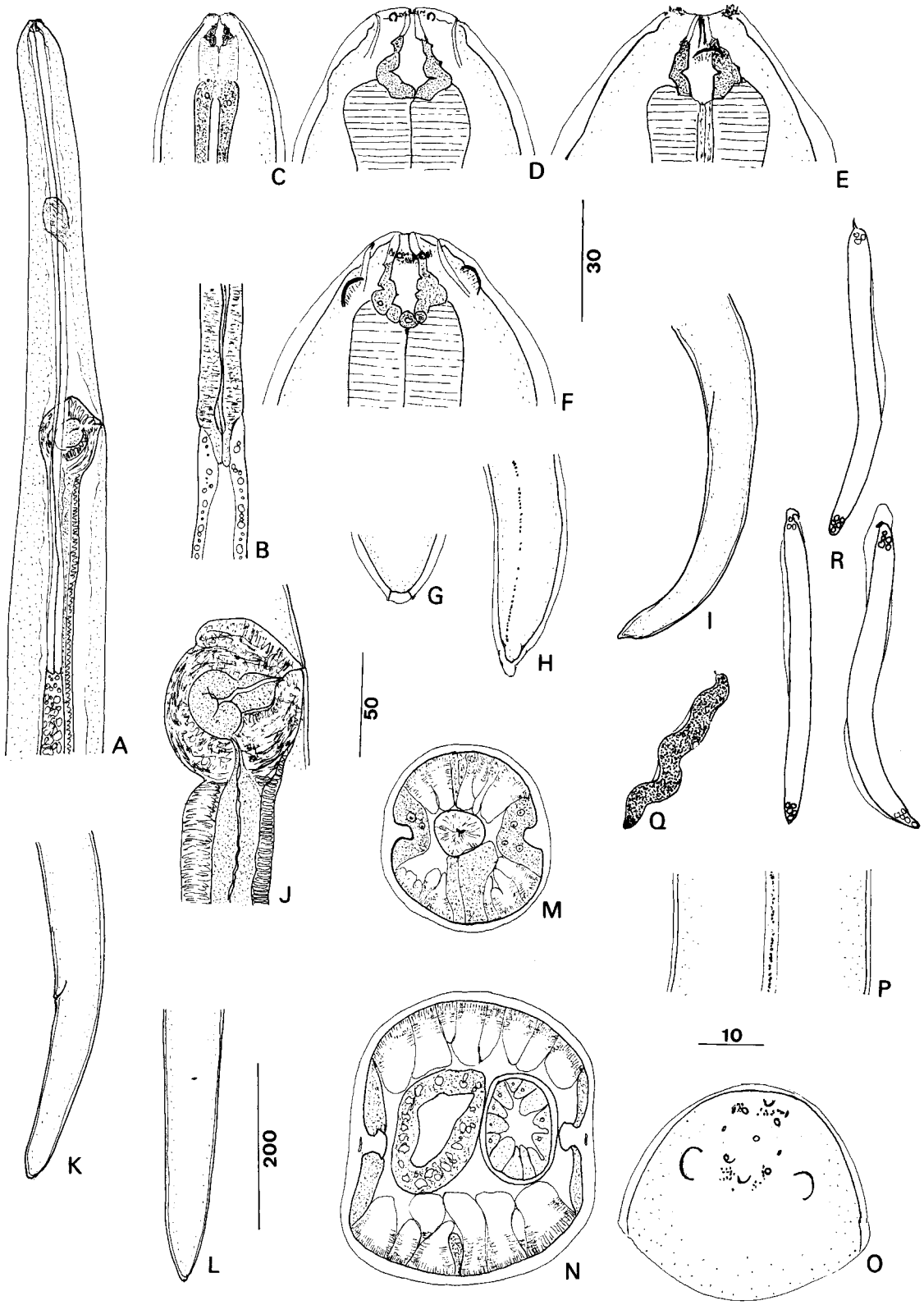

Fig. 2. – *Litomosoides yutajensis* n. sp. Female. A. Anterior region, right lateral view (allotype). B. Oesophageal–intestinal junction. C. Anterior extremity, lateral view (allotype). D. Head, dorsal view (allotype). E, F. Head, right lateral and ventral views, respectively (paratype). G. Caudal extremity, ventral view. H. Caudal ornamentation of tail, left lateral view. I. Tail, lateral view (allotype). J. Vagina, right lateral view. K, L. Tail of a paratype, left lateral and ventral view, respectively. M. Cross section at level of oesophagus. N. Cross section, posterior to vagina. O. Head, semi-apical view. P. Lateral cuticular ornamentation, 700 µm from tail. Q. Microfilaria from a thick blood smear (type host). R. Three microfilariae extracted from uteri (paratype). Scales in µm: A, I, K, L, 200; B, C, G, Q, 30; D, E, F, O, R, 10; H, J, M, N, P, 50.

composed of a cuticular axis with lateral alae, followed by a terminal membranous flap (one male was devoid of a left spicule). Right spicule uniformly cuticularized and with prominent subterminal dorsal heel. Area rugosa present. No bosses on the lateral line of the body cuticle.

Female with vulva at level of middle of the oesophagus, spherical vagina; tail straight or slightly bent ventrally, with conical extremity; a tiny tubercle near each phasmid. In the posterior mid-part of the body, cuticle ornamented with a lateral row of bosses. Mushroom-shaped internal lateral thickenings of the body cuticle, near level of oesophageal-intestinal junction.

Microfilaria. From ovijector (n = 6), 57.5 µm long (extremes 55-60 µm) and 3.7 µm wide (3.6-4 µm); body attenuated at both extremities; salient cephalic hook, posterior extremity conical, thick and nucleated; sheath of same length as the body of the microfilaria. In Giemsa-stained blood films, the microfilariae were shorter (43-48 µm) and thicker (6 µm) and a cephalic hook and sheath were often identified.

Taxonomic summary

Type host: *Pteronotus parnelli* (Gray, 1843) Mormoopidae, Noctilionoidea, Chiroptera.

Infection site: Peritoneal cavity.

Type locality/collection dates: Yutaje, Amazonas, Venezuela, 4–8/12/2002 except no. 433 SE collected in January 2000.

Specimens deposited: Holo- and alloptypes 102 CV are in the collection of the Departamento de Parasitologia, Museo de Biologia, Universitas Central de Venezuela (CP-MBUC n° 4-401201). Other specimens are deposited in the collections of the Muséum National d'Histoire Naturelle, Paris: paratypes 102 CV, other specimens 105 CV and 117 CV; slides of blood microfilariae 100 CV, 112 CV, 113 CV, 126 CV, 127 CV and 433 SE.

Biological data

In Yutaje, the bat species most frequently captured in December 2001 was *P. parnelli*, a species living in caves: 10 specimens in a total of 25 bats. The 15 other bats were *Carollia perspicillata* (Linnaeus, 1758) (n = 4), *C. castanea* H. Allen, 1890 and *C. brevicauda* (Schinz, 1821) (one specimen each), *Phyllostomus hastatus* (Pallas, 1767) (n = 3) and *P. elongatus* (Geoffroy St. Hilaire, 1810) (n = 1), *Artibeus obscurus* Schinz, 1821 (n = 3), *Tonatia saurophila* Koopman & Williams, 1951 (n = 1) and *Vampyrum spectrum* (Linnaeus, 1758) (n = 1). None were infected with filariae.

Eight of 10 *P. parnelli* were infected; two had adult worms of both sexes and blood microfilariae, one harboured three males and had no blood microfilariae, five had only blood microfilariae. *O. bacoti*, Ornithonyssinae, was fed on three microfilarial bats. Only 58 of 408 fed mites survived 15 days and, at dissection, none harboured infective larvae or any other developing larvae. The few wild mites recovered from *P. parnelli* were a different macronyssid, a new species of *Radfordiella* Fonseca, 1948 (to be described by R. Guerrero), Macronyssinae; no filarial larva was recovered at dissection.

DISCUSSION

The generic identification of the new material to *Litomosoides* is based upon the large thick buccal capsule which is posteriorly embedded in the oesophagus, and the sheathed microfilaria with a salient cephalic hook and body attenuated at both extremities (Guerrero *et al.*, 2002). The right spicule of these specimens is delicate but it is sclerotized right up to its distal extremity and the left spicule has a distal flap, showing that they belong to the *L. carinii* group. This group is represented in marsupials, rodents and bats from the New World (Bain *et al.*, 1989). In Chiroptera, however, several species were assigned to this group or to the *L. sigmodontis* group only based on the original descriptions and, as shown by Bain *et al.* (2003) for *L. hamletti* Sandground, 1934, these descriptions may be contradicted when the type materials are re-examined. Consequently the material from *P. parnelli* will be compared with all known species from bats.

No species of *Litomosoides* hitherto studied in detail has the median cephalic bosses with rugosities seen in our specimens, and all are distinct in several other characters:

The two species from marsupials (Guerrero *et al.*, 2002), *L. petteri* Bain, Petit, Berteaux, 1980 and *L. wilsoni* Guerrero, Martin, Gardner & Bain, 2002, have a tubular buccal cavity, caudal papillae in the male symmetrically arranged, a right spicule with a salient terminal cap or hook, and microfilariae with attenuated posterior extremities. In addition, *L. petteri* has eight head papillae symmetrically arranged, a longer buccal capsule, a glandular region of the oesophagus, and longer microfilariae, while *L. wilsoni* has a shorter oesophagus, a longer right spicule, and a post-oesophageal vulva.

The five species from rodents (Notarnicola & Navone, 2002) *L. carinii* (Travassos, 1919), redescribed by Bain *et al.* (1989), *L. silvai* Padhila & de Faria, 1977, redescribed by Moraes Neto *et al.* (1996) and Notarnicola *et al.* (2000), *L. scotti* Forrester & Kinsella, 1977, redescribed by Bain *et al.* (1989), *L. bonaerensis* Notarnicola, Bain & Navone, 2000, and *L. odilae* Notarnicola & Navone, 2002 have a post-oesophageal vulva, a longer right spicule (about 100 µm long) with a ter-

minal dorsal hook; each species also differs in the shape of the buccal capsule and, except for *L. carinii*, in its length (shorter for *L. scotti*, longer for the three other species). The microfilariae of *L. carinii* have not been described; those of the other species have an attenuated posterior extremity.

The 15 *Litomosoides* species and subspecies currently known from bats (Notarnicola *et al.*, 2000; Guerrero *et al.*, 2002, Bain *et al.*, 2003) are distinct from our material. Two are known only as microfilariae: those of *L. colombiensis* Esslinger, 1973 are twice as long and taper posteriorly to form a narrow tail; while those of *L. caliensis* Esslinger, 1973 have a thinner posterior extremity with two aligned caudal nuclei. *L. teshi* Esslinger, 1973, is larger, has a long thin tail in both sexes and is presently the only species which undoubtedly belongs to the *sigmodontis* group. The dimensions of *L. brasiliensis* Lins de Almeida, 1936 and *L. serpiculae* Guerrero, Martin, Gardner & Bain, 2002 are much larger and the shape of the spicules is different. The six following species: *L. leonilavasquezae* Caballero, 1939 (see also Caballero, 1944), and *L. fosteri* Caballero, 1947 (both placed by Bain *et al.*, 1989 in the *sigmodontis* group), *L. artibei* Esslinger, 1973 and *L. solarii* Guerrero, Martin, Gardner & Bain, 2002, both known by the female and its microfilariae, *L. h . hamletti* Sandground, 1934 and *L. h. penai* Jiménez-Quirós & Arroyo, 1960, both redescribed by Bain *et al.* (2003) are similar in length to our specimens but have a narrow cylindrical buccal cavity; in addition the three first species have a post-oesophageal vulva; the two last species have a right spicule with a terminal hook. *L. molossi* Esslinger, 1973, *L. chitwoodi* Bain, Guerrero & Rodriguez, 2003 (= *Litomosoides* sp. Chitwood, 1938), known as the female only and re-examined by Esslinger (1973), and *L. chandleri* Esslinger, 1973 have a lateral line of cuticular bosses in the female as do our specimens; however *L. molossi* is much smaller with a post-oesophageal vulva, a narrow tubular buccal cavity, a shorter left spicule with a relatively longer handle, two caudal lappets in the female, and longer microfilariae with attenuated posterior extremities; *L. chitwoodi* is shorter, with a post-oesophageal vulva and a very long tail (almost 600 µm); *L. chandleri* has a shorter body in both sexes, a less anterior vulva, a shorter tail in the male with only one or two pairs of caudal papillae, a right spicule with a distal cap, and microfilariae with more attenuated posterior extremities (Esslinger, 1973; Guerrero *et al.*, 2002). Finally, *L. guiterasi* (Pérez Vigueras, 1934), which resembles *L. chandleri* (*cf.* Bain *et al.*, 2003), differs from our specimens in that the body is twice as short, the vulva is near the distal extremity of the oesophagus and in the male, the tail and the left spicule are shorter. The present material is thus a new species, that we name *Litomosoides yutajensis* n. sp.

CONCLUSION

L. yutajensis n. sp. from Mormoopidae has no phylogenetically important characters which differentiate it from the species found in other Chiroptera. These species are mostly parasitic in another family of Noctilionoidea, the Phyllostomidae, with a few exceptions such as *L. molossi* from Molossidae (Vespertilionoidea) or *L. brasiliensis* from Phyllostomidae and Vespertilionidae (Vespertilionoidea) (*cf.* Lins de Almeida, 1936; Diaz-Ungria, 1963).

Filarial infection of *P. parnelli* was often revealed only by the presence of microfilariae. The persistence of this blood stage when adult worms have already been destroyed and eliminated is often questionable, but in these cases and that published concerning *L. chandleri* (Bain *et al.*, 2002) it is certain, because bats are small animals in which coelomic filariae cannot be overlooked. It is interesting to relate these observations to those made with filarial models, in which it was shown that the first larval stage, the microfilaria, seems to be controlled by different immunological mechanisms from the adult stage (Lawrence, 1996).

Attempts to obtain infective larvae of *L. yutajensis* from mites did not succeed with the two macronyssid species examined. *Radfordiella* sp. recovered from *P. parnelli* did not survive long enough to assess their vector capacity for *L. yutajensis* and, contrary to previous work with another species from a bat (Bain *et al.*, 2002), no larvae were obtained from *O. bacoti*. This could be due to the insufficiently high density of the microfilariae in blood, the instability of the temperature during postfeeding maintenance of mites, and/or the poor survival rate of the mites; alternatively, it might indicate that *O. bacoti* does not permit development of *L. yutajensis*. *O. bacoti* has not been collected from *P. parnelli* nor from any other South American bat (Saunders, 1975), whereas it was common on the terrestrial mammals which may harbour *Litomosoides* species.

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